# Screening and Separation of Microorganisms Degrading PCBs

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We performed an assay to assess the polychlorinated biphenyl (PCB) degradative capability and congener specificity of aerobic microorganisms. Microbial strains were isolated and separated from different types of soils in the Czech Republic, and their PCB-degrading abilities were compared. An industrial mixture of PCB congeners ranging from dichloro- to hexachlorobiphenyl and representing various chlorination patterns was used throughout. The PCB degradative ability of microorganisms was determined by gas chromatography after 7 days of incubation. The degree of degradation was found to depend on the number of chlorine substituents. *Key words:* biodegradation, microorganisms, PCBs, screening. *Environ Health Perspect* 102:552–554 (1994).

Metabolic breakdown of PCBs by microorganisms is considered to be one of the major routes of environmental degradation for these widespread pollutants. PCBs were used worldwide for a broad range of applications for more than 50 years (*1*). Major uses included transformer oil, capacitor dielectric fluid, heat-transfer fluid, fire retardants, and plasticizers. Their thermal and chemical stability, resistance to chemical corrosion, and general inertness have contributed to their persistence in the environment near the sites of their production, use, storage, or disposal. Because of their hydrophobic nature, PCBs have accumulated primarily in soils and aquatic sediments, where they adsorb strongly to organic matter.

Environmental contamination by PCBs was first reported in 1966. Since then, a number of PCB-degrading microorganisms have been reported (*2–4*) from studies of soils, isolates from nature, and recombinant organisms. The results indicate that the microbial aerobic degradation of PCBs leading to biphenyl ring cleavage is generally limited to less-chlorinated congeners, leaving highly chlorinated congeners unaltered (*5*). Environmental monitoring has demonstrated that the PCBs found in environmental samples are the more chlorinated biphenyls, i.e., those containing five or more chlorines per biphenyl molecule (*6,7*). The objective of this study was to find indigenous bacterial cultures capable of effective PCB degradation and to describe their properties.

## Materials and Methods

The mineral medium consisted of $(NH_4)_2SO_4$ (1 g); $Na_2HPO_4 \cdot 7H_2O$ (8.2 g); $KH_2PO_4$ (2.7 g;) $MgSO_4 \cdot 7H_2O$ (0.1 g); $Ca(NO_3)2$ (0.5 g); and $FeSO_4 \cdot 7H_2O$ (0.25 g), dissolved in 1000 ml of distilled water. The pH was adjusted to 7.2 with 1 M NaOH before autoclaving. The liquid complex medium was Luria broth, consisting of Pepton (10 g), yeast extract (5 g), and NaCl (10 g). The solid medium was Luria broth medium solidified by 20 g/l of agar. The inoculation medium was mineral medium supplemented by 100–200 mg/l of biphenyl, dissolved in 5 ml of acetone. The assay medium was mineral medium supplemented by 15–140 mg/l of Delor 103, dissolved in 5 ml of acetone.

All flask cultivations were performed on a rotary shaker (140 turns per min), placed in a dark, temperate box. Temperature was kept at 28°C. The flasks were closed with cotton wool and covered with aluminum foil.

Soil samples from the surface and 50 cm underneath the surface were collected from the PCB-contaminated area in Rozmital pod Tremsinem. We transferred 10 g of these samples into 250-ml sterile Erlenmeyer flasks containing 100 ml of Luria broth medium. After 3 days of cultivation on a rotary shaker, 10 ml of the supernatant from each soil sample was transferred into 250-ml Erlenmeyer flasks containing 100 ml mineral medium with biphenyl as the sole carbon source and 10 ml of the Luria broth culture was used as inoculum for the assay medium with Delor 103 (see Table 2).

Another enrichment procedure used a forest topsoil collected from a recreational mountain area in a northern region of the Czech Republic. It did not contain any detectable amount of PCBs nor any other chlorinated compounds likely to interfere with the analysis of PCBs. We mixed 100 g of this soil with Delor 103 (150 ppm) and biphenyl (1500 ppm). This mixture was exposed to the effect of PCBs under laboratory conditions for 3 months. We kept the soil in a dark box under an ambient temperature. Water content was periodically restored by adding sterile water. At the end of the incubation period, the same procedure used for contaminated soil was employed.

We poured 0.1 ml of each supernatant onto petri dishes with 12 ml of complex solid medium to obtain microbial strains. After 24 hr of cultivation at 28°C, the single colonies were separated. For the first testing, 30 Gram-negative and Gram-positive strains, including sporeforming microbial strains, were used. Single colonies grown after 24 hr were transferred into 100 ml of mineral medium with 20 mg biphenyl in 0.5 ml acetone as the sole carbon source and cultivated on the rotary shaker under the standard conditions described above. We tested the surviving bacterial strains for their PCB-degrading ability. All strains used for the biodegradative experiments in this paper were Gram-negative rods.

To prepare the inoculum, all tested strains and cocultures were pregrown for 48 hr on 100 ml mineral medium with 10 mg biphenyl under conditions described above.

Either 10 ml of liquid inoculum of the microbial coculture or 10 ml of single-culture inoculum for testing the PCB degrading ability of pure microbial culture was added to the 100 ml of mineral medium with Delor 103 (1.5–14 mg/100 ml, added as a solution in 0.5 ml of acetone) as the sole carbon source and transferred into sterile 250-ml Erlenmeyer flasks. The duration of cultivation on a rotary shaker at 28°C was 7 days. Afterwards the flasks were treated in a water bath (80–100°C) for 10 min.

We stopped incubation of 100 ml PCB-degrading cultures by heating them at 80–100°C for 10 min and then extracted them with 20 ml hexane at 20°C on a shaker for 1 hr. We diluted 10 µl of hexane layer with hexane to a final volume of 10 ml.

Samples were analyzed on a Hewlett-Packard 5890 gas chromatograph with an electron capture detector and a fused silica capillary column (30 m, 0.20 mm inner diameter) coated with 0.25 µm immobilized phase of SE-54 and nitrogen as a carrier gas (flow rate 1 ml/min). The temperature program was 50°C for 1 min, then 25°C/min until 180°C, 4°C/min until 280°C, then isothermically. The sample amount injected was 2 µl [(splitless, according to Grob and Grob (*8*)]. All of the Delor 103 congeners were identified by comparing their retention times with the retention times of corresponding peaks of Aroclor 1242 (with known congeners) (*9*). Table 1 contains 31 chromatographic peaks from the complete list of 59 separate Delor 103 peaks, whose area was larger than 0.3% of the total area of all chromatographic peaks

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**Table 1.** Assignment of congeners to peaks of Delor 103 analyzed on gas chromatography-electron capture detector

| Peak no.[a] | Ballschmitter[b] | Substitution |
|---|---|---|
| 1 | 5, 8 | 2, 3; 2, 4' |
| 2 | 19 | 2, 6, 2' |
| 3 | 18 | 2, 5, 2' |
| 4 | 17, 15 | 2, 4, 2'; 4, 4' |
| 5 | 16, 32 | 2, 3, 2'; 2, 6, 4' |
| 6 | 26 | 2, 5, 3' |
| 7 | 25 | 2, 4, 3' |
| 8 | 31 | 2, 5, 4' |
| 9 | 28 | 2, 4, 4' |
| 10 | **33**, 53 | 3, 4, 2'; 2, 5, 2', 6' |
| 11 | **22**, 51 | 2, 3, 4; 2, 4, 2', 6' |
| 12 | 45 | 2, 3, 6, 2' |
| 13 | 52 | 2, 5, 2, 5 |
| 14 | 49 | 2, 4, 2', 5' |
| 15 | 47 | 2, 4, 2', 4' |
| 16 | 48 | 2, 4, 5, 2' |
| 17 | 44 | 2, 3, 2', 5' |
| 18 | 37, 42 | 2, 4, 4'; 2, 3, 2', 4' |
| 19 | 41, 64, 71 | 2, 3, 4, 2', 2, 3, 6, 4'; 2, 6, 3', 4' |
| 20 | 40 | 2, 3, 2', 3' |
| 21 | 74 | 2, 4, 5, 4' |
| 22 | 70 | 2, 5, 3', 4' |
| 23 | 66, 95 | 2, 4, 3', 4'; 2, 3, 6, 2', 5' |
| 24 | 56, 60 | 2, 3, 3', 4'; 2, 3, 4, 4' |
| 25 | 84, 92 | 2, 8, 6, 2', 3'; 2, 3, 5, 2', 5' |
| 26 | 90, 101 | 2, 3, 5, 2', 4'; 2; 4, 5; 2', 5' |
| 27 | 97, 152 | 2, 4, 5, 2', 3'; 2, 3, 5, 6, 2', 6' |
| 28 | 87 | 2, 3, 4, 2', 5' |
| 29 | 85 | 2, 3, 4, 2', 4' |
| 30 | 110, 77 | 2, 3, 6, 3', 4'; 3, 4, 3', 4' |
| 31 | 149, 118 | 2, 3, 6, 2', 4', 5'; 2, 4, 5, 3', 4' |

[a]Array of Delor 103 peaks identified in our chromatographic system
[b]Numbers refer to Ballschmitter (*10*) enumeration. Congeners in boldface type are major components of the peak.

(*10*). The relative area of the peaks of samples were calculated by matching the area of peaks that were degraded with the area of the same peaks in a blank sample (without bacterial culture).

## Results and Discussion

### Variables Affecting Quantitative Recovery of PCBs

The volatility, hydrophobicity, and insolubility of PCBs pose particular difficulties in aqueous biodegradative assays and require special attention. Physical loss of PCBs due to evaporation or adsorption to the inoculation vessel and the bacterial cells has often been mistakenly attributed to biodegradation. To minimize these problems, we designed an assay in which the inoculation and extraction of each sample were carried out in a single Erlenmeyer flask. The commercial PCB product Delor 103 is found in the majority of contaminated sites in the Czech Republic, thus it was used to test the biodegradative capabilities of microorganisms.

### Limitations of Biodegradation Assays with Commercial PCB Mixtures

There are several disadvantages to using Delor 103 assays to measure degradation of PCBs. Delor mixtures (as well as Aroclors) are produced by direct chlorination of biphenyl, and each usually consists of more than 40 detectable PCB congeners in differing proportions. The concentrations of individual congeners are often not known and cannot be inferred from visual inspection of a GC profile obtained with an electron capture detector, since response factors for congeners with the same number of chlorines can vary as much as eightfold. Despite these facts, we developed an assay that overcame the majority of these limitations and that screened microorganisms for the ability to degrade PCB. Several investigators (*2,3,11*) have noted that the pattern of chlorination may influence biodegradation.

The measure of biodegradative activity for individual congeners is the remaining amount of the congener that can be expressed as the ratio between the area of specific peak after biodegradation to the area of the same peak in the blank experiment. Using standard conditions both for the preparation of the sample for analysis and for gas chromatography, the accuracy of the results obtained may be influenced by the efficiency of the extraction, as well as the arrangement of the blank experiment. The influence of these two parameters was evaluated before starting this study (data not shown). Possible variants of blank experiments are as follows: 1) standard solution of Delor 103 in hexane, 2) mineral medium and Delor 103 without microorganisms, 3) mineral medium with added microbial inoculum treated for 10 min in a 80–90°C water bath, after it has been mixed with Delor 103, 4) mineral medium with microbial inoculum and Delor 103, after 30 min of Delor 103 addition followed by 10 min in a 80–90°C water bath, 5) mineral medium with microbial inoculum, the usual cultivation, followed by thermal treatment and the subsequent addition of Delor 103, 6) mineral medium with microbial inoculum, the usual cultivation, then addition of Delor 103, and, after 30 min of Delor 103 addition, thermal treatment.

The experimentally verified time of 1 hr was sufficient for an extraction in all of the above-mentioned variations. Recovery was 90–110%, and experimental error was the same for all Delor 103 congeners and was not influenced by the prolongation of an extraction. For the sake of maximal operational simplicity the second alternative and an 1-hr extraction time were chosen.

**Table 2.** Degradation of Delor 103 by the cocultures[a]

| | Remaining PCB (%) after biodegradation | | |
|---|---|---|---|
| | Type of coculture | | |
| Peak no. | I | II | III |
| 1 | 70 | 102 | 60 |
| 2 | — | — | — |
| 3 | 80 | 100 | 66 |
| 4 | 81 | 99 | 65 |
| 5 | 83 | 100 | 70 |
| 6 | 92 | 95 | 89 |
| 7 | — | 102 | — |
| 8 | 94 | 100 | 80 |
| 9 | 95 | 100 | 81 |
| 10 | 93 | 98 | 80 |
| 11 | 97 | 95 | 86 |
| 12 | 93 | 100 | 79 |
| 13 | 98 | 97 | 81 |
| 14 | 99 | 97 | 83 |
| 15 | 100 | 95 | 81 |
| 16 | — | — | — |
| 17 | 97 | 97 | 86 |
| 18 | 97 | 97 | 88 |
| 19 | 100 | 99 | 85 |
| 20 | 89 | 87 | 76 |
| 21 | 105 | 98 | 93 |
| 22 | 104 | 98 | 94 |
| 23 | 104 | 98 | 95 |
| 24 | 104 | 97 | 94 |
| 25 | 101 | 97 | 82 |
| 26 | 106 | 102 | 130 |
| 27 | — | — | — |
| 28 | — | — | — |
| 29 | — | — | — |
| 30 | 104 | 100 | 99 |
| 31 | 81 | 74 | 78 |

[a]Inocula for this degradation was subcultured on Luria broth medium.

**Table 3.** Effect of precultivation on biphenyl on the degradation of Delor 103 by the cocultures

| | Remaining PCB (%) after biodegradation | | |
|---|---|---|---|
| | Type of coculture | | |
| Peak no. | I | II | III |
| 1 | 57 | 50 | 32 |
| 2 | 50 | 51 | 32 |
| 3 | 61 | 56 | 35 |
| 4 | 61 | 54 | 35 |
| 5 | 62 | 53 | 36 |
| 6 | 66 | 56 | 40 |
| 7 | 65 | 52 | 37 |
| 8 | 69 | 57 | 41 |
| 9 | 68 | 55 | 41 |
| 10 | 68 | 56 | 42 |
| 11 | 73 | 64 | 48 |
| 12 | 66 | 57 | 38 |
| 13 | 70 | 58 | 43 |
| 14 | 71 | 58 | 43 |
| 15 | 70 | 58 | 42 |
| 16 | 70 | 60 | — |
| 17 | 66 | 90 | 42 |
| 18 | 71 | 60 | 46 |
| 19 | 71 | 56 | 45 |
| 20 | 83 | 57 | 45 |
| 21 | 77 | 55 | 51 |
| 22 | 78 | 55 | 53 |
| 23 | 79 | 54 | 51 |
| 24 | 77 | 56 | 52 |
| 25 | 82 | 65 | 67 |
| 26 | 86 | 57 | — |
| 27 | 76 | — | — |
| 28 | 79 | 60 | 52 |
| 29 | 75 | 60 | 50 |
| 30 | 82 | 58 | 56 |
| 31 | 78 | 59 | 58 |

## Degradation of Delor 103 by Mixed Bacterial Cultures

The Delor 103 mixture was used to test three mixed bacterial cultures, as well as several single bacterial strains derived from PCB-contaminated sites. Tables 2 and 3 show Delor 103 degradation by coculture mixtures with different histories. Most of the mixed cocultures used degrade only a few congeners, generally the less chlorinated ones. The difference in PCB-degrading ability between these two experiments is significant. Table 2 presents the results of the biodegradation obtained from the three bacterial mixtures isolated as described above, but without inoculation on biphenyl (10 ml Luria broth coculture mixture in 100 ml mineral medium was used). This experimental arrangement was not successful: the cultures had practically no degradative competence (at the same concentration of Delor 103—6 mg/100 ml mineral medium). The effect of precultivation on mineral medium with biphenyl on the biodegradation of Delor 103 is shown in Table 3.

Bacterial mixed culture III, isolated from a soil sample contaminated with Delor 103 under laboratory conditions, seemed to possess the best PCB degradative potency; the less chlorinated the congeners, the smaller the remaining amount of PCB.

## Degradation of Delor 103 by Individual Bacterial Strains

We isolated 30 bacterial strains from the liquid phase of the enrichment culture and incubated them on media with biphenyl, as described earlier. The bacterial growth was checked on petri plates after 36, 48, and 72 hr of cultivation. We chose those bacterial strains capable of growth and tested them for their biodegradative competence. Table 4 presents the individual bacterial strains 1/2 and 1/4 that were isolated from the contaminated soil surface. The great difference between their PCB-degradative potency is evident. Strain 1/4 had a great range of degradative ability and different congener specificity. Dichlorobiphenyls and trichlorobiphenyls (except 2,4,4', and 3,4,2', respectively) are practically degraded. But the amount of 2,2',3',4,5-pentachlorobiphenyl or 2,2',3,5,6,6'-hexachlorobiphenyl was not changed. The 1/2 strain attacked the Delor 103 congeners as well, but it is evident that this strain has a much smaller range of PCB-degradative ability overall.

Individual cultures 2/2, 2/3, and 2/5 were isolated from the contaminated soil, 50 cm under the surface. The comparison among these cultures and 1/4 and 1/2 strains is remarkable. Cultures 2/2 and 2/3 contain practically no PCB-degradation properties. Culture 2/5 provided better degradation of some congeners (pentachlorobiphenyls and hexachlorobiphenyls) than the 1/4 culture did.

Table 4 also presents data obtained with bacterial cultures isolated from artificially contaminated soil samples. Culture 3/1 seems to be the best member of this group from the point of view of its extensive degradative ability.

We found two individual strains (1/4 and 3/1) capable of degrading a rather wide range of PCB congeners, isolated by different means from the soil samples. Both strains should be studied further for their characteristics, as they are promising for use in biodegradation.

**Table 4.** Degradation of Delor 103 by individual cultures

| | Remaining PCB (%) after biodegradation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cultures[a] | | | | | | | |
| Peak no. | 1/2 | 1/4 | 2/2 | 2/3 | 2/5 | 3/1 | 3/2 | 3/4 |
| 1 | 73 | 0.1 | 81 | 98 | 44 | 3 | 52 | 89 |
| 2 | 64 | 0 | 69 | 103 | 39 | 0 | 44 | 86 |
| 3 | 76 | 8 | 82 | 104 | 48 | 5 | 57 | — |
| 4 | 79 | 0 | 86 | 104 | 49 | 5 | 56 | 96 |
| 5 | 77 | 7 | 86 | 104 | 45 | 3 | 20 | 96 |
| 6 | 78 | 4 | 85 | 103 | 49 | 8 | 57 | 98 |
| 7 | 82 | 0 | 30 | 99 | 18 | 9 | 60 | 3 |
| 8 | 81 | 11 | 88 | 99 | 52 | 9 | 60 | 100 |
| 9 | 81 | 11 | 86 | 97 | 55 | 9 | 60 | 98 |
| 10 | 83 | 15 | 87 | 99 | 56 | 11 | 70 | 100 |
| 11 | 82 | 40 | 89 | 110 | 56 | 8 | 55 | 107 |
| 12 | 81 | 0 | 89 | 109 | 71 | 12 | 59 | 103 |
| 13 | 80 | 13 | 92 | 105 | 71 | 12 | 61 | 103 |
| 14 | 81 | 13 | 87 | 106 | 57 | 14 | 62 | 104 |
| 15 | 86 | 13 | 87 | 106 | 91 | 13 | 60 | 103 |
| 16 | 83 | 0 | 92 | 103 | 61 | 13 | 62 | 99 |
| 17 | 85 | 24 | 91 | 103 | 56 | 15 | 64 | 104 |
| 18 | 88 | 32 | 92 | 98 | 62 | 34 | 63 | 104 |
| 19 | 87 | 24 | 94 | 101 | 62 | 12 | 50 | 106 |
| 20 | 71 | 27 | 94 | 104 | 49 | 20 | 64 | 108 |
| 21 | 90 | 33 | 76 | 98 | 70 | 20 | 64 | 108 |
| 22 | 88 | 40 | 92 | 98 | 68 | 21 | 64 | 106 |
| 23 | 89 | 41 | 92 | 98 | 68 | 22 | 65 | 106 |
| 24 | 92 | 60 | 95 | 97 | 69 | 23 | 68 | 115 |
| 25 | 98 | 60 | 99 | 109 | 78 | 30 | 71 | 122 |
| 26 | 84 | 59 | 80 | 104 | 66 | 13 | 40 | 122 |
| 27 | 150 | 104 | 55 | 108 | 40 | 20 | 46 | 127 |
| 28 | 46 | 87 | 68 | 115 | 55 | 18 | 40 | 120 |
| 29 | 74 | 91 | 65 | 114 | 74 | 24 | 56 | 111 |
| 30 | 86 | 125 | 81 | 99 | 62 | 33 | 73 | 126 |
| 31 | 102 | — | 100 | 99 | 50 | — | — | — |

[a]All these individual cultures were Gram-negative rods.

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